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The isotopic composition of respired carbon dioxide in scleractinian corals: Implications for cycling of organic carbon in corals

Peter K. Swart[1,*], Alina Szmant[2], James W. Porter[3], Richard E. Dodge[4], Jennifer I. Tougas[3], and John R. Southam[5]

[1]Division of Marine Geology and Geophysics, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA
[2]Center for Marine Science, University of North Carolina at Wilmington, 5600 Marvin Moss Lane, Wilmington, NC 28409, USA
[3]Institute of Ecology, University of Georgia, Athens, GA 30602, USA
[4]National Coral Reef Institute, Nova Southeastern University, Dania, FL, USA
[5]Department of Geological Sciences, University of Miami, Coral Gables, FL 33129, USA

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Abstract—The origin of $\delta^{13}C$ variations within the skeletons of zooxanthellate scleractinian corals is still a matter of considerable debate. In particular, the role respired CO_2 plays in controlling the eventual $\delta^{13}C$ of the skeleton remains unclear. In this study, the temporal variability of the $\delta^{13}C$ of respired CO_2 produced by *Montastraea faveolata* has been measured at approximately monthly intervals over a 1-year period. In these experiments, three corals maintained on a platform at 8 m depth near Molasses Reef in the Florida Keys were incubated in closed chambers for 24-h periods and samples of the incubation water analyzed for the $\delta^{13}C$ of the dissolved inorganic carbon (ΣCO_2) at ~3-h intervals. Throughout the incubation, the concentration of O_2 was measured continuously within the chamber. Our results show that during daylight, the $\delta^{13}C$ of the ΣCO_2 in the incubation water becomes enriched in ^{13}C as a result of fractionation during the fixation of C by photosynthesis, whereas at night the $\delta^{13}C$ of the ΣCO_2 becomes more negative. The $\delta^{13}C$ of the respiratory CO_2 ranges from −9‰ in the late spring to values as low as −17‰ in the autumn. The lighter values are significantly more negative than those reported by previous workers for coral tissue and zooxanthellae. An explanation for this discrepancy may be that the corals respire a significant proportion of isotopically negative substances, such as lipids, which are known to have values up to 10‰ lighter compared to the bulk $\delta^{13}C$ of the tissue. The clear seasonal cycle in the $\delta^{13}C$ of the respiratory CO_2 suggests that there is also seasonal variability in either the $\delta^{13}C$ of the coral tissue or the type and/or amount of organic material being respired. A similar temporal pattern and magnitude of change was observed in the $\delta^{13}C$ of the coral tissue samples collected from a nearby reef at monthly intervals between 1995 and 1997. These patterns are similar in timing to the $\delta^{13}C$ measured in the coral skeletons. We have also calculated an annual mean value for the fractionation factor between dissolved CO_2^- in the external environment and photosynthate fixed by the zooxanthellae of 1.0121 (±0.003). This value is inversely correlated with the ratio of photosynthesis to respiration (P/R) of the entire organism and shows the highest values during the summer months.

1. INTRODUCTION

There have been numerous papers which have attempted to explain the origin of carbon isotopic variations within the skeleton of scleractinian corals (Weber, 1970; Swart, 1983; McConnaughey, 1989, 2003; Swart et al., 1996; Grottoli and Wellington, 1999). The leading hypothesis at present is that skeletal $\delta^{13}C$ responds to variation in solar insolation and kinetic effects, with more positive $\delta^{13}C$ values occurring under conditions of high insolation and presumed higher rates of photosynthesis and slower rates of skeletal precipitation. In addition, recent studies (Adkins et al., 2003) have proposed that the $\delta^{13}C$ and $\delta^{18}O$ of nonzooxanthellate coral skeletons are related to the pH at the site of calcification following the suggestion of Zeebe (1999) as applied to foraminifera. Under such a mechanism, high pH levels which would promote high rates of calcification also cause negative $\delta^{13}C$ and $\delta^{18}O$ values. Such a model is contrary to the commonly applied theory in which isotopically negative $\delta^{13}C$ and $\delta^{18}O$ are believed to be a result of kinetic factors (McConnaughey, 1989, 2003). This model has been recently applied to zooxanthellate corals (Rollion-Bard et al., 2003).

The positive relationship between insolation and the $\delta^{13}C$ values of coral skeletons is believed to be a result of the fractionation of CO_2 during incorporation by photosynthetic endosymbiotic algae which are found in many corals (zooxanthellae) (Goreau, 1977). Hence, during higher rates of photosynthesis there is greater discrimination against $^{13}CO_2$, which preferentially accumulates in the internal CO_2 pool involved in calcification. In attempts to test this hypothesis, various workers have grown corals under controlled light and temperature conditions and measured the $\delta^{13}C$ of the deposited skeleton (Weil et al., 1981; Swart et al., 1996; Juillet-Leclerc et al., 1997; Grottoli and Wellington, 1999; Reynaud-Vaganay et al., 2001). Results from these experiments have generally confirmed the hypothesis that increasing insolation results in higher $\delta^{13}C$ values in the coral skeleton. However, in the only experiment in which the ratio of photosynthesis to respiration (P/R) was measured during the period of skeletal accretion, an inverse correlation was observed between skeletal $\delta^{13}C$ and P/R, opposite to that expected (Swart et al., 1996). Increases in the rate of skeleton formation have been suggested to result in more negative $\delta^{13}C$ and $\delta^{18}O$ values (McConnaughey, 1989).

* Author to whom correspondence should be addressed (pswart@rsmas.miami.edu).

In this model, slow rates of equilibration between CO_2 and HCO_3^- at the sites of calcification impede isotopic equilibrium. Hence portions of the coral skeleton with low rates of accretion possess $\delta^{13}C$ and $\delta^{18}O$ values close to the theoretical equilibrium.

In contrast to numerous studies on the $\delta^{13}C$ of skeletal material, there have been relatively few reports on the $\delta^{13}C$ of organic material in scleractinian corals (tissue and zooxanthellae). The studies which do exist have investigated the bulk $\delta^{13}C$ of coral organic material, separated zooxanthellae, and coral tissue as a function of depth (Land et al., 1975; Muscatine et al., 1989) and locality (Heikoop et al., 2000). Muscatine et al. (1989) found that in shallow dwelling corals, the $\delta^{13}C$ of the coral tissue was similar to that of the zooxanthellae (−10 to −12‰). With increasing water depth, the $\delta^{13}C$ of the coral tissue and zooxanthellae became increasingly dissimilar with the coral tissues becoming more negative and approaching the $\delta^{13}C$ of zooplankton (−20 to −22‰). This change was interpreted as reflecting a high amount of translocation of organic compounds from the zooxanthellae to the coral in shallow water compared to deeper dwelling corals (Muscatine et al., 1989). Deeper water corals are therefore interpreted to rely more on heterotrophy compared to shallower water corals. Changes in the $\delta^{13}C$ with depth have been also noted in the skeleton. Typically the skeletons of shallow water corals have more positive $\delta^{13}C$ values than deeper water corals (Weber et al., 1976; Land et al., 1977; Erez, 1978; Swart and Coleman, 1980). These changes have been interpreted as reflecting a decrease in the rate of photosynthesis with increasing water depth.

Common to all previous studies on the $\delta^{13}C$ of coral tissues is the fact that these studies represent samples collected during only one particular time period of the year. There has been no recognition that the $\delta^{13}C$ of coral tissues and zooxanthellae might have some temporal variability. This study attempts to examine potential temporal variability in the $\delta^{13}C$ of the respired CO_2, coral tissue, and zooxanthellae which might eventually contribute to or influence the $\delta^{13}C$ of the coral skeleton.

2. EXPERIMENTAL APPROACH

The experiments reported in this paper were conducted between May 1993 and April 1994 at a location close to Molasses Reef in the Florida Keys, U.S.A. (Fig. 1). Eighteen, 4-inch-diameter cores of *Montastraea faveolata* were drilled from massive colonies from several different nearby reefs at approximately the same water depths. Care was taken to minimize damage to living tissues during the drilling process. These cores were placed in PVC tubes which were fixed to a plastic platform cemented into the reef rock at a depth of 8 m. Before the experiments started, the corals were left to acclimatize for several months. At approximately monthly intervals, all of the corals were removed from the PVC tubes, cleaned of any adhering algae, and placed in clean plastic tubing so that only the live coral tissue was exposed. All corals were left for 24 h to recuperate before the start of any incubations. The incubations took place in respirometers previously described in Porter (1980) (Fig. 2). Initially the respirometers had a chamber volume of 1.7 L, but this was increased to 2.9 L after the first 3 months (see "Results" and "Discussion" sections). The chamber was continually stirred during the incubation using magnetic stirrers, and the concentration of oxygen and light was monitored. Data were continuously recorded by a data recorder located in the base of the equipment (Fig. 2). After 2- to 3-h periods, 60 mL of the water were extracted by divers using syringes for the measurement of alkalinity and pH. After each incubation, the chambers were completely flushed with new seawater by removing the chamber from the coral and allowing new seawater to circulate around the coral. The procedure was then repeated every 3 h for a 24-h period. For corals 1, 2, and 3 (previously collected from Pickles Reef, Fig. 1), water samples were also taken for the analysis of the $\delta^{13}C$ of the ΣCO_2 at the start and at the end of each incubation. These samples were passed through a 0.5-μm filter, spiked with $HgCl_2$, and maintained in sealed glass ampules until they were analyzed. Coral skeletons were stained using Alizarin red-S (15 mg/L for 8 h) at the end of the incubation period to leave a stain line in the skeleton for the purposes of calibration of drilled samples to absolute time. Skeletal material from corals 2 and 8 were analyzed for their skeletal $\delta^{13}C$ and $\delta^{18}O$ values. Specimen 8 was analyzed in addition to coral 2 as it showed a higher rate of linear extension and therefore enabled a higher sample resolution to be obtained during the experimental period. These two corals were chosen as they exhibited the widest range of extension rates and therefore allowed an assessment of the impact of growth rate upon the $\delta^{13}C$ and $\delta^{18}O$ values of the skeleton. In addition to these experiments, samples of coral tissue were collected from corals at Pickles Reef (Fig. 1) between 1995 and 1997. These were analyzed for the $\delta^{13}C$ of the animal tissue and zooxanthellae. Coral tissues were not analyzed from the incubated individuals as this would have severely stressed the corals.

2.1. Alkalinity and pH

Water from the syringes was filtered through a GF/A glass fiber filter into acid-washed vials. Ten cm^3 of this sample was pipetted into small shell vials which fit into a thermally controlled titration chamber. Samples were titrated using 0.01 N HCl with a computer-controlled Brinkman titrator interfaced with an Orion pH meter and Ross electrode, and the alkalinity was determined using a Gran titration method (Stumm and Morgan, 1996). The pH was calculated using the initial reading on the pH meter before the start of the titration. The pH was calibrated using the NBS scale. The change in the CO_3^{2-} concentration of the water was determined using the change in pH, and alkalinity and the stoichiometric equilibrium (*K) constants of Millero (2000). All pH and alkalinity measurements were performed within 30 min of sample collection.

2.2. Oxygen

The concentration of oxygen was determined using a polarographic oxygen probe calibrated in the conventional manner. Data were recorded every 4 min on a data logger contained within the respirometers (Fig. 2).

2.3. Light

Light levels were recorded continuously at the same depth as the incubations (8 m) using a LiCor 4 π spherical quantum sensor.

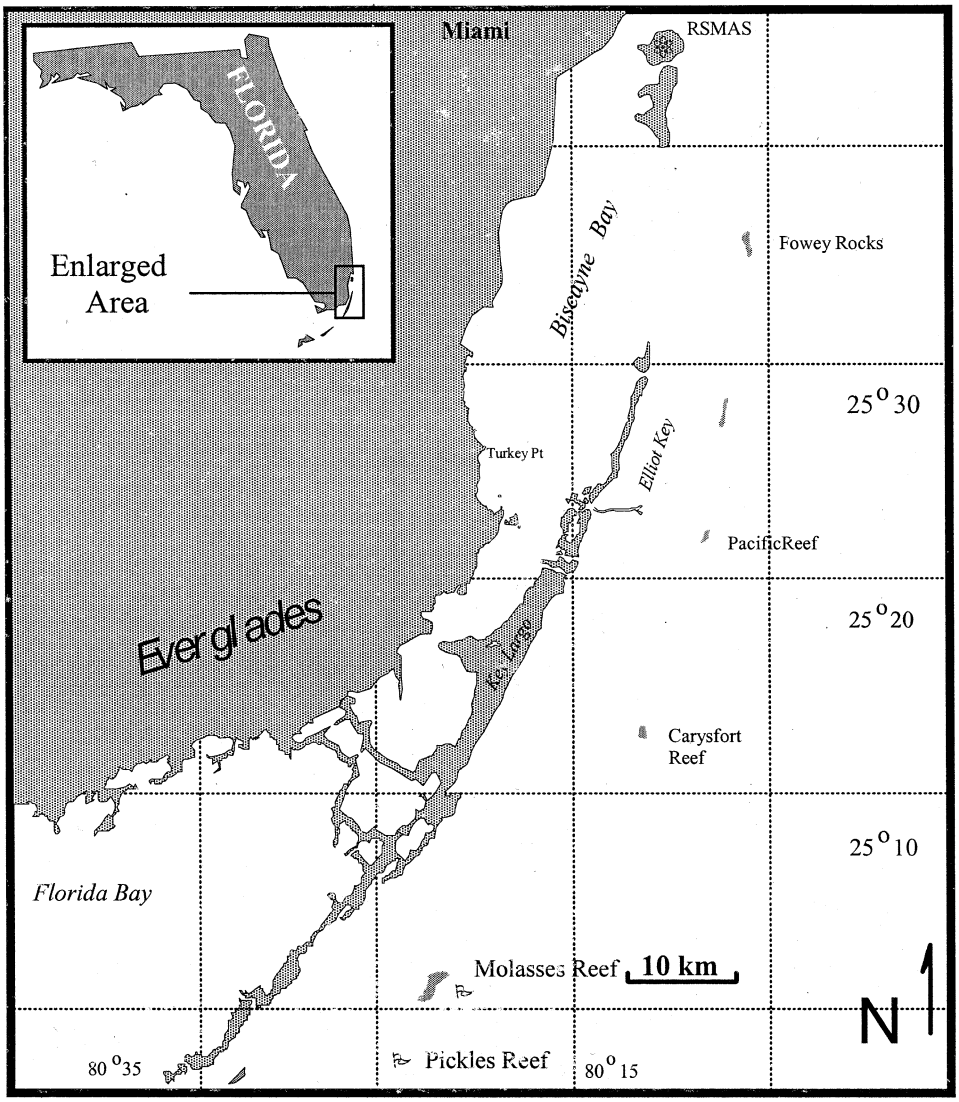

Fig. 1. Map showing location of Molasses and Pickles Reefs.

2.4. Temperature

Two Ryan thermographs were attached to the platform where the corals were housed. The thermographs were shielded inside large-diameter, open-ended PVC tubes to prevent a greenhouse effect during daytime hours.

2.5. Coral Tissues

Samples of live coral skeleton with tissue were collected at approximately monthly intervals from the sides of colonies of *Montastraea faveolata* growing between 3 and 8 m water depth on Pickles Reef (Fig. 1) between March 1995 and May 1997. This is the same locality from which corals 1, 2, and 3 were collected before being transplanted to Molasses Reef. Samples were collected from different colonies throughout the experiment in an effort to minimize damage to individual coral colonies. Tissues were removed from the coral skeleton by air-brushing (Szmant et al., 1989) within 24 h of collection, and the $\delta^{13}C$ was determined on the separated zooxanthellae and tissue samples. Tissue slurries were homogenized for 30 s using an Ultra-Torrax homogenizer, and the homogenate was centrifuged for 3–5 min in a table-top centrifuge at 1500 rpm. The supernatant (animal fraction) was decanted and frozen for later analysis. The pellet was resuspended three times in filtered sea water, and any contaminating skeletal fragments were allowed to settle out and removed before recentrifuging. The final pellet was recovered with small volumes of deionized water and frozen for later isotopic analysis. Before analysis, the organic tissues were washed in dilute HCl to remove any adhering carbonate material, rinsed in distilled water, and dried in a low-temperature oven. Analyses of the $\delta^{13}C$ of organic C were performed using an automated C/N analyzer linked to a continuous-flow stable isotope mass spectrometer (Europa 20-20). Data are corrected for the conventional interferences and reported relative to Vienna Pee Dee belemnite (V-PDB) as parts per thousand in the conventional notation. Each sample was

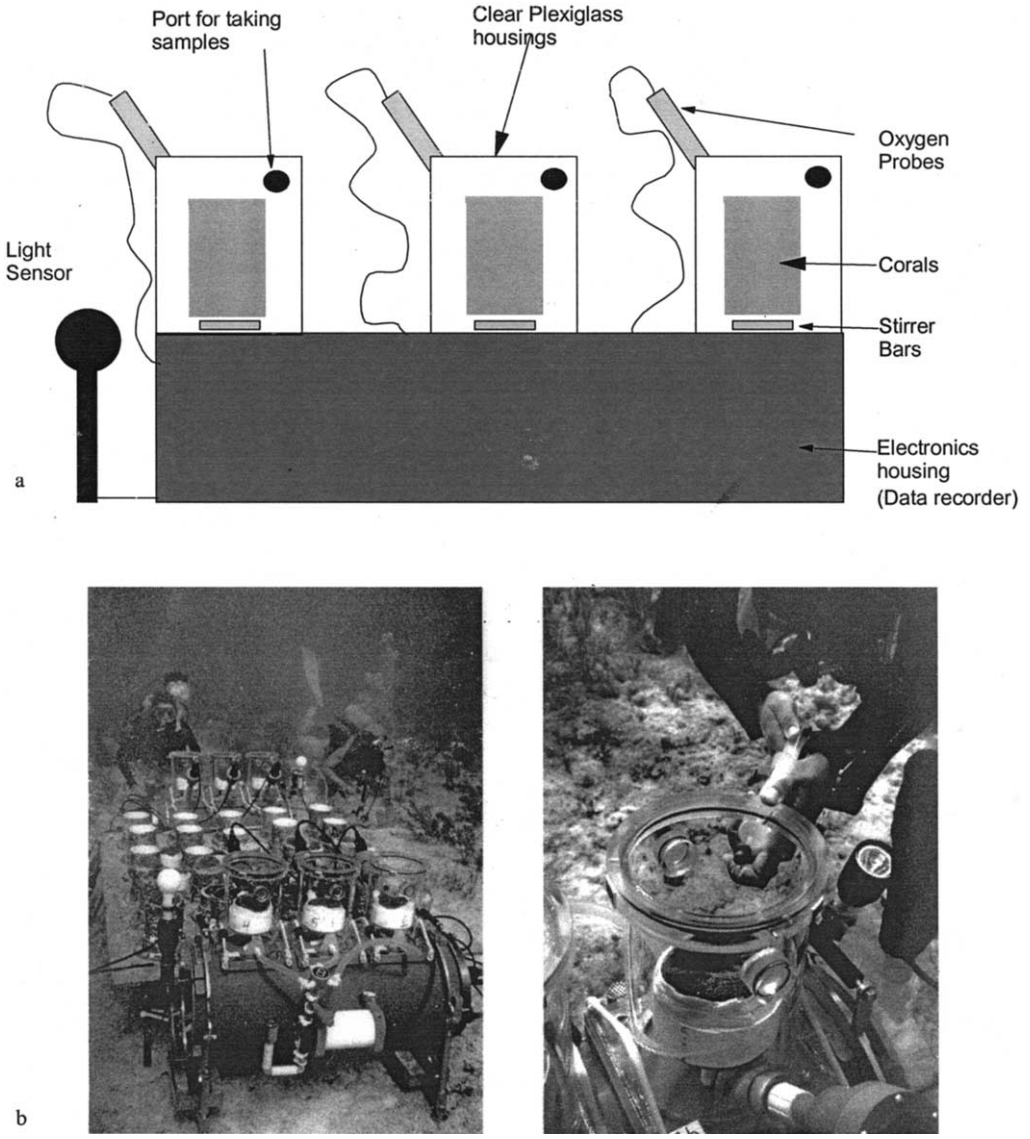

Fig. 2. (a) Schematic of incubation chambers. (b) Close-up of incubation chambers with experimental coral in place. The platform on which the corals are maintained during experiments is shown in the background. On the right hand the diver is extracting a sample for measurement of the $\delta^{13}C$ of the ΣCO_2 through a port in the incubation chamber.

analyzed in triplicate. Typical standard deviation determined on multiple analyses of standard material is 0.1‰.

2.6. Carbon Isotopic Measurements

The $\delta^{13}C$ of the seawater ΣCO_2 was determined on CO_2 released by acidification under vacuum. Standardization was achieved by measuring the $\delta^{13}C$ of CO_2 released from a 4 mM sodium bicarbonate solution, the $\delta^{13}C$ of which had been previously determined by conventional methods. The gas was analyzed using a stable isotope mass spectrometer (Finnigan-MAT 251), and the standard deviation of 0.1‰ was determined through multiple analyses of standards. Data are reported as deviations from the initial $\delta^{13}C$ of the ΣCO_2 and are normalized to the volume of the chamber and the time of each incubation.

2.7. Carbonate Materials

Samples were excised from the coral skeleton using a computer-controlled microsampling device from the two corals that showed the highest and lowest growth rates during the experimental period (corals 2 and 8). For coral 8, the samples were drilled with a home-built, computer-controlled device, and the powder generated was analyzed using a common acid bath attached to a Finnigan-MAT 251. For coral 2, the samples were drilled using a New Wave microsampling device and were analyzed using an automated carbonate device (Kiel III) attached to a Finnigan Delta plus stable isotope mass spectrometer. Results from the two instruments are directly comparable as the same internal standards were used. One standard deviation (1 SD) of standards for both instruments are typically better than 0.08‰ for $\delta^{18}O$ and 0.05‰ for $\delta^{13}C$.

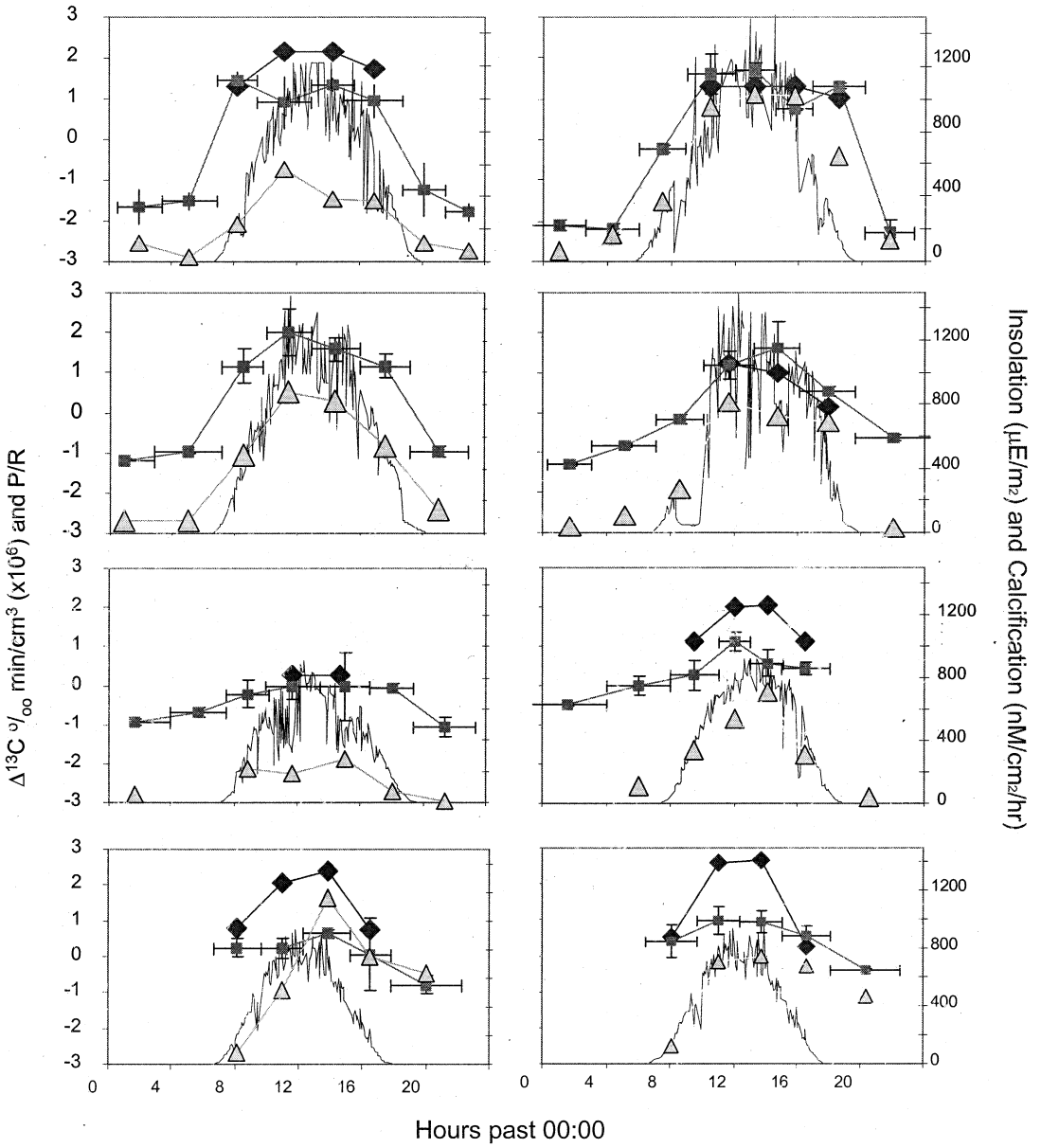

Fig. 3. (a–h) Changes in the carbon isotopic composition of the chambers (squares) normalized to time and volume for the months May (a), June (b), July (c), August (d), September (e), October (f), December (g) 1993 and February (h) 1994. The change in the $\delta^{13}C$ of the ΣCO_2 is divided by the number of minutes and the volume of each chamber. Horizontal error bars represent the period of the incubation, and vertical error bars represent ± 1 SD of the carbon isotopic measurements made on the chambers housing corals 1, 2, and 3. If no vertical error bars are seen, then the error is smaller than the symbol. Superimposed on each graph is a 30-min running mean of the light data (line without symbols) in $\mu Em^{-2}s^{-2}$ recorded at each chamber. Also shown on each graph is the ratio of photosynthesis to respiration (P/R) (diamonds) and rate of calcification (triangles). The scale for the light and the calcification is given on the right hand *y*-axis.

3. RESULTS

3.1. Incubation Experiments

For each incubation, the $\delta^{13}C$ of the ΣCO_2 of water surrounding the corals (1, 2, and 3) was measured before and after the incubation. The mean change in $\delta^{13}C$ of the ΣCO_2 and standard deviation of this change was calculated using the data from these three incubations. The $\delta^{13}C$ data are presented in Figures 3a–h as changes in the carbon isotopic composition from the initial $\delta^{13}C$ value of ΣCO_2 normalized to the length of incubation, the volume of the chamber ($\Delta\ \delta^{13}C$), and the mean surface area of the corals. The surface area ranged between 80 and 85 cm^2 for the three corals and did not change within the error of the measurement during the course of the experiment.

The $\delta^{13}C$ of the initial seawater ΣCO_2 exhibited considerable variation on a seasonal basis, with values becoming more positive during April–June 1993 and then more negative during August and September. Changes in the $\delta^{13}C$ of the initial ΣCO_2

during the diurnal cycle were small. The causes for diurnal changes are complex and are beyond the scope of this paper, but relate to the timing of ebb tide relative to the daylight hours. Similar changes in the $\delta^{13}C$ of the ΣCO_2 were noted by Weber and Woodhead (1971) in reefs from Florida. Generally speaking, all incubations exhibited similar patterns in the changes of the $\delta^{13}C$ of the ΣCO_2 of the incubation water. During the daylight hours, the $\delta^{13}C$ of the ΣCO_2 in the chamber increased, and during the nighttime hours the $\delta^{13}C$ values became more negative. The only exception to this pattern was observed in September 1993, when no enrichments were observed during the daytime incubations, although the $\delta^{13}C$ values became more negative during the nighttime. During July 1993, dark incubations were carried out during daylight hours by using blackened chambers. The magnitude of depletion in $\delta^{13}C$ during this darkened incubation was similar to that observed at nighttime (Table 1).

3.2. Oxygen

Changes in oxygen consumption and production are shown in Table 1. Changes in the ratio of photosynthesis to respiration (P/R) during the daytime are shown in Figure 3. During the initial 3 months, when chamber volumes of 1.7 L were used, it was noticed that oxygen concentrations in the closed volumes became very high (>10 ppm; Table 1) during the daytime. High levels of oxygen, such as observed with the smaller volume chambers, which were above supersaturation, could cause gas to come out of solution as well as possibly increase the rate of respiration during the day relative to night and perhaps promote photorespiration. To remedy this potential problem, the volume of the chambers was increased from 1.7 to 2.9 L, starting with the August 1993 incubation. Despite the increase in the chamber volume, the maximum respiration rates measured in the smaller chambers were not significantly different compared to the larger chambers, although the concentrations of O_2 during the nighttime dropped to ~2 ppm in the smaller chambers compared to 2.5 ppm in the larger ones (Table 1). Oxygen data from July 1993 were lost as a result of an instrument malfunction.

3.3. Alkalinity and Calcification

The alkalinity decreased in response to calcification by the animals. Generally there were decreases during all the incubations, indicating some degree of calcification even during the nighttime (Table 1 and Fig. 3). Calcification and extension rate were also measured using the optical density of the skeleton combined with extension rates measured from X-radiographs (Dodge et al., 1994). The mean extension rates during the experimental period for corals 1, 2, and 3 were lower (2.4–5.3 mm/yr) for this particular species than those measured by other workers (Hudson, 1981) (Fig. 4). In contrast, other corals that went through the same treatment showed more normal growth rates, and coral 8 which was analyzed for its C and O isotopic composition, showed a growth rate of 7.3 mm/yr. This rate is similar to those previously measured for this species in Florida.

3.4. Skeletal Material

The $\delta^{13}C$ of the skeletal material varied from −3 to −0.5‰ (Fig. 5a). The most negative values occurred during the late summer (September to October, 1993) and the most positive values between February to April, 1994. There was no statistically significant variations in the mean $\delta^{13}C$ of the skeleton between corals 2 and 8 despite large variation in the skeletal growth rates. The changes in the oxygen isotopic composition of the skeletal material were consistent with previously published relationships between temperature and $\delta^{18}O$ (Leder et al., 1996) (Fig. 6). The change in $\delta^{18}O$ in our study was 1‰ for every 4.6°C in temperature, as compared to 4.5°C in the studies by Leder et al. (1996) using *Montastraea annularis*, a sibling species related to *M. faveolata* (Knowlton et al., 1991).

3.5. Coral Tissues

The $\delta^{13}C$ of the coral tissue, sampled 2 yr after our experimental incubations from the same reef where the corals were collected, showed a temporal variation, increasing from values of approximately −13.5‰ early in the year to −11‰ in May 1995 (Fig. 5c). A similar pattern was repeated the following year although the amplitude of the change in $\delta^{13}C$ of the coral tissues was not as great. The $\delta^{13}C$ of zooxanthellae were more variable and did not exhibit a clear intra-annual variation (Table 2).

4. DISCUSSION

4.1. Explanation for Changes Observed in the Incubation Chambers

Changes in the $\delta^{13}C$ of the ΣCO_2 in the incubation chamber during the course of the experiments are a function of (i) the addition of isotopically negative respiratory CO_2 from the host coral and zooxanthellae, (ii) fractionation during the uptake of CO_2 by photosynthesis, and (iii) precipitation of skeletal material. As fixation of carbon by the zooxanthellae takes place principally during the daylight hours, when insolation is available, changes in the $\delta^{13}C$ of the ΣCO_2 at night can be ascribed only to respiration and calcification. There has also been some reported fixation of carbon in the absence of light in corals (Cook, 1983) which might influence the magnitude of the $\delta^{13}C$ depletion at night. However, we believe that this effect is likely to be minor (see later discussion). Hence, during the nighttime the entire changes in the $\delta^{13}C$ of the ΣCO_2 in the incubation water is a result of the addition of CO_2 from respiration, and fractionation of the ΣCO_2 pool by calcification. By knowing the amount of oxygen consumption and the amount of calcification that has taken place, the $\delta^{13}C$ of the respiratory CO_2 can be calculated during a particular incubation. An additional parameter that has been employed in calculations of this kind is the respiratory quotient (RQ). The RQ is defined as the number of moles of oxygen needed to oxidize 1 mol of organic material (Parsons et al., 1977). The RQ value depends upon the nature of the organic material which is oxidized. For example, the oxidation of 1 mol of carbohydrate requires ~1 mol of oxygen and would therefore have a RQ of 1. In contrast, a complex fatty acid would have a RQ of 1.4. A similar quotient can be calculated for the process of photo-

Table 1. Summary of physiological and isotopic data. Values represent the mean of the values obtained from the three corals that were incubated.

Month	Finishing time	Carbon $\Delta\ \delta\ ^{13}C$	Carbon (1 σ) $\Delta\ \delta\ ^{13}C$	$\Delta\ \delta\ ^{13}C$ ‰ cm^{-3} $m^{-1} \times 10^6$	ΔO_2 O_2 (mg O_2/L)	Max. O_2 ppm	Calcification nMol/cm^2/h	Calcification Std dev.
May 1993	3:29	−0.50	0.38	−1.65	−5.15	2.81	26.0	37.0
	6:55	−0.53	0.08	−1.52	−5.86	2.47	232.0	17.6
	9:26	0.38	0.04	1.46	1.37	6.59	570.3	60.7
	12:58	0.33	0.23	0.92	7.06	10.20	388.3	78.2
	15:42	0.38	0.04	1.34	5.99	10.05	376.7	60.4
	18:46	0.36	0.15	0.94	3.58	8.81	117.0	24.1
	21:30	−0.34	0.18	−1.23	−4.48	4.02	70.0	43.8
	0:00	−0.55	0.06	−1.78	−4.91	3.19	111.3	68.3
June 1993	2:41	−0.73	0.04	−2.10	−5.95	1.99	169.3	48.6
	6:02	−0.75	0.05	−2.21	−6.08	1.86	370.3	87.5
	8:57	0.07	0.03	−0.24	−0.65	5.01	950.7	41.3
	11:59	0.49	0.15	1.61	7.78	10.04	1025.0	103.1
	14:31	0.44	0.05	1.70	7.36	10.02	1020.3	102.2
	16:54	0.19	0.02	0.77	6.59	9.63	644.3	62.0
	20:10	0.43	0.02	1.30	0.94	6.27	130.0	44.5
	23:17	−0.72	0.09	−2.26	−5.91	1.86	70.0	22.1
July 1993	2:49	−0.79	0.00	−1.18	nm	nm	78.7	66.5
	7:13	−0.75	0.01	−0.98	nm	nm	486.0	97.6
	9:55	0.54	0.11	1.16	nm	nm	879.3	38.3
	12:52	1.00	0.17	2.00	nm	nm	821.0	69.0
	16:00	0.27	0.09	1.59	nm	nm	540.0	99.3
	19:10	0.65	0.09	1.17	nm	nm	142.7	30.7
	22:58	−0.64	0.05	−0.97	nm	nm	81.3	33.6
Dark chamber	10:55	−0.60	0.08	−1.52	nm	nm	44.3	23.8
August 1993	3:07	−0.62	0.01	−1.30	−7.73	2.59	107.3	43.9
	7:08	−0.59	0.02	−0.83	−8.20	2.47	272.0	56.6
	10:01	−0.09	0.06	−0.18	−3.58	4.11	815.3	35.4
	13:17	0.68	0.20	1.18	6.88	7.72	724.0	41.7
	16:09	0.80	0.33	1.61	5.83	7.50	683.3	195.0
	19:38	0.32	0.08	0.53	0.70	5.55	32.7	26.9
	0:30	−0.49	0.07	−0.65	−7.89	2.64	41.7	30.0
September 1993	3:55	−0.73	0.07	−0.92	−8.59	2.10	−48.3	53.7
	7:29	−0.42	0.07	−0.67	−6.73	2.75	214.3	18.0
	10:19	−0.10	0.17	−0.21	−1.27	4.64	188.0	47.1
	13:26	−0.01	0.19	−0.02	0.66	5.19	279.3	4.0
	16:37	−0.11	0.32	−0.02	0.88	5.61	68.7	22.3
	19:25	−0.03	0.07	−0.06	−2.71	4.63	12.7	18.2
	23:22	−0.73	0.16	−1.06	−8.03	2.54	51.0	20.1
October 1993	04:16	−0.42	0.02	−0.48	−6.72	2.98	112.3	124.4
	07:59	−1.35	0.16	0.00	−7.12	3.32	341.0	144.4
	11:02	0.14	0.21	0.26	−4.95	7.03	540.0	199.7
	13:05	0.39	0.08	1.12	4.63	7.73	712.3	164.8
	15:10	0.21	0.12	0.58	5.59	7.85	308.7	187.7
	18:03	0.23	0.09	0.43	5.39	7.64	44.3	12.3
	23:02	−0.43	0.02	−0.48	4.67	3.39	−53.3	19.2
December 1993	9:39	0.13	0.13	0.25	3.43	6.70	520.3	18.4
	12:18	0.11	0.12	0.24	9.88	9.11	1159.7	325.4
	15:16	0.33	0.06	0.64	10.41	9.51	745.3	30.9
	17:47	0.03	0.44	0.07	2.95	6.86	637.7	43.7
	22:16	−0.61	0.20	−0.78	−7.40	3.05	82.3	69.6
February 1994	9:42	0.22	0.25	0.39	2.25	6.72	703.7	70.0
	12:25	0.46	0.51	0.98	9.33	8.94	748.3	9.5
	15:08	0.44	0.32	0.93	9.99	9.33	676.3	126.2
	18:08	0.30	0.54	0.57	1.11	6.60	466.7	172.1
	22:35	−0.30	0.28	−0.39	−7.35	3.94	126.7	65.8
April 1994	13:42	nm	nm	nm	3.63	7.12	297.3	40.7
	16:55	nm	nm	nm	4.65	6.39	363.7	90.1
	19:45	nm	nm	nm	1.12	4.19	313.3	45.9
	22:45	nm	nm	nm	−4.85	4.42	31.3	24.1
	4:25	nm	nm	nm	−3.48	4.34	nm	nm
	6:57	nm	nm	nm	−2.28	4.36	nm	nm
	10:25	nm	nm	nm	2.89	6.72	337.3	61.4

nm = not measured.

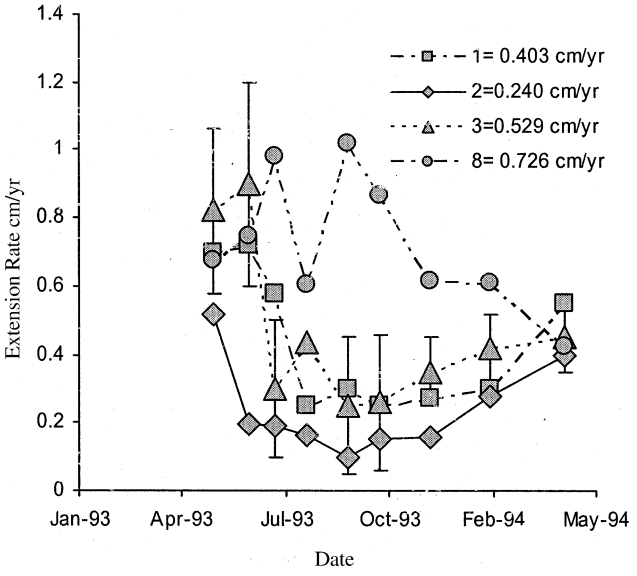

Fig. 4. Extension rates for corals used in this study. Rates have been determined by the measurement of linear extension along five transects in the coral skeleton and are reported as mm/yr for each interval. The error bars for coral 3 represent ±1 SD of the five extension measurements made on that coral. Other error bars are not shown for clarity, but are similar to those shown for coral 3. Note that coral 8 has an extension rate which is approximately 5 times higher than that in coral 2.

synthesis (PQ). As the types of compounds which are being oxidized and being produced are not known, we have for modeling purposes assumed a RQ and PQ of unity. Potential implications of the variation in this parameter will be discussed later. Assuming that the rate of respiration is more or less the same throughout a 24-h period, the daylight change in the $\delta^{13}C$ of the ΣCO_2 can be modeled as a combination of respiration, calcification, and photosynthesis. Rates of calcification are calculated using the change in the alkalinity and photosynthesis by the change in the oxygen corrected for the rate of respiration. It has been shown that the assumption of a constant rate of respiration throughout a diurnal cycle may not be valid (Edmunds and Davies, 1988) and higher rates may occur during the daytime. Implications of a higher daytime respiration rate are discussed later.

4.2. Respiratory CO_2

The $\delta^{13}C$ of the respired CO_2 can be calculated by using the amount of oxygen consumed during the dark using the following equation:

$$R_m = (1 - y)R_i + yR_r \quad (1)$$

In this equation, the R_m = the $^{13}C/^{12}C$ ratio of the ΣCO_2 measured at the end of the incubation, the R_i = $^{13}C/^{12}C$ ratio of of the ΣCO_2 in the water, and the R_r = $^{13}C/^{12}C$ ratio of the HCO_3^- in equilibrium with the CO_2 added by respiration. Although some calcification was measured during the nighttime (Table 1), for the initial calculation it can be assumed that the rate is small. The parameter (y) is the fraction of the ΣCO_2 derived from respiratory sources (both coral animal and plant respiration). To determine (y) we used the changes in the $\delta^{13}C$ that occurred during the nighttime and calculated the amount of respiratory CO_2 added. Based on this calculation, we have determined the $\delta^{13}C$ of the respiratory CO_2 during each incubation period. Our results show that the $\delta^{13}C$ of the respired CO_2 changes on a seasonal basis, with the most isotopically negative values being produced in September through December (Fig. 5 and Table 3). Although the experiment was only carried out over a 12-month period, the most positive values occurred early in the year, between May and June 1993. We do not think that the more positive values of the respired CO_2 during this time period were related to the smaller volume chambers used in May and June 1993, as more positive values were also observed in February 1994 when the larger chambers were used. The change in the $\delta^{13}C$ of the respired CO_2 is probably

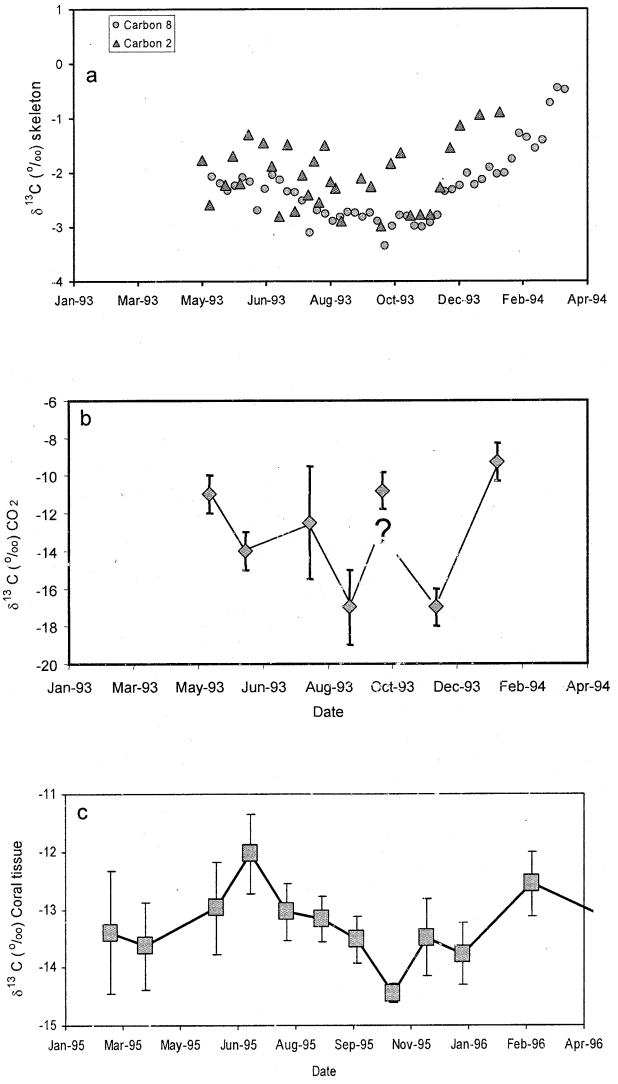

Fig. 5. (a) Changes in the $\delta^{13}C$ of the skeletal material from corals 2 and 8; the timing of each sample was estimated using stain lines (see Fig. 4). (b) Calculated $\delta^{13}C$ of the respiratory CO_2; error bars reorient 1 SD of the estimate using the standard deviation of the change in the $\delta^{13}C$ from the three experiments. (c) The $\delta^{13}C$ of coral tissue from Pickles Reef collected between 1995 and 1996; the error bars represent 1 SD of the analysis of at least four samples. Note that the time periods for (a) and (b) are different from (c).

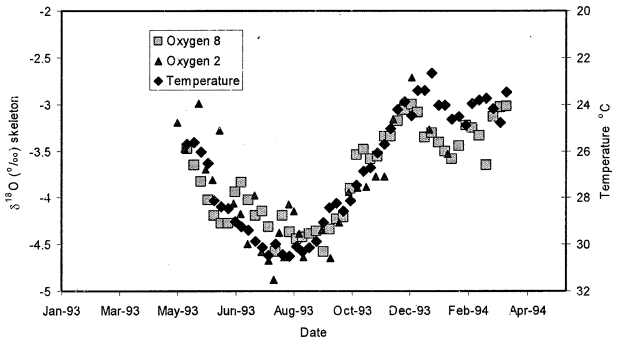

Fig. 6. Changes in the oxygen isotopic composition and temperature during the experimental period as measured in the skeleton of corals 2 and 8. The slope of the correlation between $\delta^{18}O$ and temperature is similar to previously published values (Leder et al., 1996).

best explained by changes in the type of organic material being respired and could be accommodated for by a changing RQ value (see previous discussion).

4.3. Light and Dark Modeling

Interpretation of changes in the $\delta^{13}C$ of the ΣCO_2 of the incubation water during the daytime is more complicated than during nighttime incubation as the processes of photosynthesis and calcification both influence the $\delta^{13}C$ of the ΣCO_2 within the incubation chamber. During photosynthesis, ^{13}C is preferentially discriminated against and the remaining CO_2 becomes isotopically more enriched. The discrimination is described by the parameter α_i which relates the $^{13}C/^{12}C$ ratio in the photosynthate to the $^{13}C/^{12}C$ ratio in the ΣCO_2. The discrimination encompasses a range of physiologic and kinetic processes that influence the CO_2 as it passes from the external environment into the zooxanthellae and is incorporated into the photosynthate. As values for α_i are typically greater than unity, the $\delta^{13}C$ of the ΣCO_2 becomes isotopically more positive during the course of the incubation.

Formation of the coral skeleton also causes carbon isotopic fractionation, and although the precise factors involved are not known, the coral skeletons are typically more negative than the ambient ΣCO_2 whereas inorganic aragonite is isotopically more positive than the ambient ΣCO_2 (Romanek et al., 1992). However, the eventual $\delta^{13}C$ of the coral skeleton is the sum of many different processes, and therefore for modeling of the influence of calcification upon the isotopic composition of the internal carbon pool, we shall assume the fractionation of carbon isotopes relative to the internal carbon pool (α_{ii}) is similar to that observed for the formation of inorganic aragonite (1.0026; Rubinson and Clayton, 1969). Therefore, in contrast to photosynthesis, formation of skeletal material in a closed volume results in progressively more negative $\delta^{13}C$ values for the ΣCO_2 with increasing incubation time. Although these two processes have fractionation factors which tend to cancel each other out, not only is the magnitude of the fractionation factor for photosynthesis greater than for precipitation of aragonite, but also the flux of carbon associated with photosynthesis is greater than calcification. Therefore, with increasing time the $\delta^{13}C$ of the ΣCO_2 becomes progressively more positive during the daytime. The processes that occur during the daylight incubation can be described by the time variability (t) of the six carbon concentrations ($^{12}C_{DIC}(t), ^{13}C_{DIC}(t), ^{12}Co_r(t), ^{13}Co_r(t), ^{12}C_{Sk}(t), ^{13}C_{Sk}(t)$). Conservation of mass requires that Eqn. 2 is satisfied. In the case of the closed system experiments described in this paper, Eqn. 3 is required. For example, in these equations V_1C_1 = the mass of dissolved inorganic carbon (DIC), V_2C_2 = organic carbon (Or), and V_3C_3 = skeletal material (sk) formed during the incubation. The term J describes the rates of change of the carbon reservoirs as a result of photosynthesis, respiration, and skeleton formation; e.g., $^{13}J_{ps}$ is the rate at which $^{13}C/^{12}C_{DIC}$ is increasing as a consequence of the photosynthetic process. For a more detailed development of these equations, see Appendix 1.

$$\frac{d}{dt}V_i^{\gamma}C_i = \sum_{j=1}^{3} {}^{\gamma}J_j \quad \gamma = 12 \text{ or } 13 \tag{2}$$

$$\sum_{j=1}^{3} \frac{d}{dt}V_j^{\gamma}C_j = 0 \tag{3}$$

4.4. Calculation of the Fractionation Factor

To determine the value of α_i, we assumed that the rate of respiration during the daytime was similar to that observed during the nighttime. We then calculated the amount of O_2 produced during photosynthesis and hence the amount of CO_2 fixed using a PQ value of unity. Values were substituted into Eqns. A1 to A6 (Appendix 1) and solved using numerical methods. These equations can also be used to solve Eqn. 1 by assuming a zero rate of photosynthesis. The α_i values calculated using equations shown in Appendix 1 show a mean value of 1.0121 ($\pm$0.0031). In fact, the α_i value shows a distinct seasonal variation increasing from 1.0089 in May to 1.0166 in August, before decreasing to values of between 1.0070 and 1.0097 in December and February of the following year. No significant variation in α_i was observed between the different chamber sizes.

Table 2. Mean $\delta^{13}C$ values of coral tissues and zooxanthellae from Pickles reef collected between 1995 and 1996.

	Coral tissue		Zooxanthellae	
Date	$\delta^{13}C$	1 σ	$\delta^{13}C$	1 σ
Mar-95	−13.89	0.51	−14.43	1.81
Apr-95	−13.63	0.75	−13.69	0.82
Jun-95	−12.98	0.80	−11.57	0.98
Jul-95	−12.04	0.69	−11.23	1.85
Aug-95	−13.04	0.39	−10.33	1.77
Sep-95	−13.17	0.39	−9.28	0.57
Oct-95	−13.52	0.40	−12.61	0.53
Nov-95	−14.44	0.17	Sample lost	
Dec-95	−13.49	0.67	−11.56	2.14
Jan-96	−13.77	0.54	−12.60	0.39
Mar-96	−12.56	0.46	Sample lost	
May-96	−13.13	0.54	−11.64	0.73
Jun-96	−12.66	0.85	−12.10	1.25
Sep-96	−13.06	1.11	−12.60	0.84
Oct-96	−13.39	0.63	−11.84	1.89
Nov-96	−13.33	0.61	−11.29	3.26

Table 3. Summary of estimated $\delta^{13}C$ of CO_2 and initial DIC and fractionation factor (α_1) relative to ΣCO_2 as well as light and P/R ratio.

Month	Mean light Einstein/m^2/day	P/R	α	$\delta^{13}C$ CO_2	$\delta^{13}C$ DIC
May 1993	44.70	1.43	1.0089	−11.0	1.71
June 1993	47.85	1.90	1.0102	−14.0	1.86
July 1993			nm		1.93
August 1993	43.75	1.06	1.0166	−12.5	1.44
September 1993	25.25	0.52	1.0140	−17.0	0.65
October 1993	29.40	1.66	1.0130	−10.8	1.27
December 1993	25.65	1.83	1.0070	−17.0	1.49
February 1994	19.80	1.70	1.0097	−9.3	1.27
April 1994	40.20	1.50	nm	nm	nm

nm = not measured.

The variation in the value of α_i measured in our experiments is actually a combination of a number of fractionation factors involving the uptake of carbon species from seawater into the coral. These include diffusion or movement of bicarbonate from the outer seawater into the body cavity of the coral, diffusion of CO_2 into the endodermal cells, and diffusion of bicarbonate through the intercellular spaces. Separate α values could in fact be specified for each of these processes if sufficient information were available. These factors may vary throughout the year and are related to other physiologic activities of the coral and the endosymbiont zooxanthellae.

The calculated fractionation factors provide information regarding the presence or absence of photorespiration in zooxanthellae. Photorespiration is the malfunctioning of photosynthesis in the presence of oxygen. If photorespiration were present in corals, then significant differences would be expected in the fractionation between the smaller volume incubation chambers in which oxygen levels reached significantly higher values (12 ppm) compared to the larger volume chambers (8 ppm). However, no significant differences in the change of the $\delta^{13}C$ of the ΣCO_2 during the daytime were observed between June 1993 and August 1993, suggesting that either the increased concentration of oxygen was insufficient to induce photorespiration or that photorespiration does not occur in zooxanthellate corals.

Our calculations of α_i have not taken into account the suggestion by Edmunds and Davies (1988) that rates of respiration may actually be higher during the day than at night. Hence values of α_i would be minimum estimates as higher rates of respiration would add isotopically negative carbon to the system. If daytime respiration rates are higher than those measured during the dark, then this would have the effect of increasing the estimate of α_1. For example, in the case of the May 1993 experiment, a doubling of the respiration rate would increase α_1 from 1.0089 to 1.0135.

4.5. Dark Fixation

Previous workers have suggested the possibility that some fixation of CO_2 occurs during the nighttime (i.e., dark fixation; Cook, 1983). Although data from our experiments are not definitive on this subject, one might expect that dark fixation would occur immediately after the removal of light from the system and hence the dark incubations that occurred immediately after the removal of insolation would show a lower depletion in $\delta^{13}C$ than those incubations that occurred in the middle of the night. However, in our experiments there was no continual depletion in the $\Delta\delta^{13}C$ of the ΣCO_2 in the chambers during the nighttime with $\Delta\delta^{13}C$ values being essentially identical just after dark and continuing at the depleted value throughout the night (Fig. 3a–h). In addition, the change in the $\delta^{13}C$ of the ΣCO_2 during the one dark respiration experiment which we carried out in the daytime during July 1993 produced a similar $\Delta\delta^{13}C$ of the ΣCO_2 to that observed in the middle of the night. Hence our data suggest that in these corals there was no fixation of CO_2 in the absence of light.

4.6. Variations in the Carbon Isotopic Composition of the Coral Tissues and Respired CO_2

Although tissue samples were not taken from the coral during the incubation period, results from these analyses indicate there is seasonal variation in the $\delta^{13}C$ of the tissue with the most isotopically negative values occurring during summer months (Fig. 5c). The $\delta^{13}C$ of the zooxanthellae were quite variable and did not show a clear seasonal pattern (Table 2). Although the changes in $\delta^{13}C$ of the coral tissue between months are in most instances statistically significant, the range of values within each month may have been affected by the fact that the samples were not collected from the same individual coral colony. Hence, part of the inter- and intramonthly variation is probably related to sample-to-sample variability. As coral tissue and zooxanthellae have differing $\delta^{13}C$ values, it is possible that part of the intermonthly variability arises from cross-contamination during the sample preparation procedure. However, previous work using the same method has shown that there is only a small amount of zooxanthellae in the coral tissue (less than 5%; Fitzgerald and Szmant, 1997), and we therefore consider this to be unlikely. In contrast, there can be significant contamination of the zooxanthellae by the coral tissue during separation (up to 50%; Fitzgerald and Szmant, 1997). Therefore, although the variability in the zooxanthellae data might be explained by variable amounts of host-tissue contamination, it is unlikely that the same could hold true for the seasonal changes in the carbon isotopic composition of the coral tissue.

4.6.1. Chemical composition of the coral tissues

One possible explanation for the seasonal variations in the $\delta^{13}C$ of the coral tissues and the respired CO_2 is that, as previously proposed by Szmant et al. (1989), corals are respiring a large proportion of lipids in addition to other organic

compounds. Lipids are known to have significantly more negative carbon isotopic compositions than other biochemical components (Abelson and Hoering, 1961; Galimov, 1974). This fractionation is associated with the formation of acetyl-coenzyme A, the precursor for fatty acid biosynthesis (Monson and Hayes, 1980, 1982). The result is that lipids are typically significantly depleted in the ^{13}C relative to other organic compounds. Although there have as yet been no studies on the $\delta^{13}C$ of lipids extracted from corals, it is probable that the lipid component in corals is also isotopically more negative than the bulk tissues. This finding is in agreement with other studies which have determined that lipids are the principal respiratory substance for corals (Patton et al., 1977; Crossland et al., 1980; Szmant et al., 1989).

If lipids are being respired, then the R/Q of unity must be adjusted to a slightly higher value (see previous discussion). An R/Q of 1.2, for example, would imply respiration of 50% lipid and 50% carbohydrate. As the model used for calculating respiratory CO_2 necessitates a knowledge of the R/Q, changing this value means that more organic carbon can be oxidized for a given amount of O_2, and therefore raising the R/Q from 1 to 1.2 would also act to produce a $\delta^{13}C$ of the respiratory CO_2 closer to that of the coral tissues and zooxanthellae. The $\delta^{13}C$ for the respired CO_2 calculated using this value would be closer to that typically measured in bulk coral tissue. Hence respiration of lipid acts to increase the R/Q and decrease the $\delta^{13}C$ of the respired CO_2. Both of these processes lead to a lower calculated value for the $\delta^{13}C$ of the respired CO_2. Therefore, under experimental conditions in which the $\delta^{13}C$ of the bulk tissues was measured in conjunction with the $\delta^{13}C$ of the respired CO_2, the R/Q could be determined and hence the type of organic material metabolized by the coral could be established. This was not possible in this study as no $\delta^{13}C$ measurements were performed on the coral tissues during the experimental period.

4.6.2. Autotrophy and heterotrophy

Most shallow water zooxanthellate corals rely heavily on substances translocated from the zooxanthellae for their energy requirements and are therefore considered to be autotrophic (Muscatine et al., 1981). There have been only a few studies which have examined whether the corals are autotrophic throughout the year, or whether the corals derive less of their energy needs from their zooxanthellae during particular times of the year. Porter (1985) determined that specimens of *Montastraea annularis* showed higher P/R ratios during the summer than winter, implying a greater reliance on heterotrophic sources during the winter. Clearly there is opportunity for variation in the amount of autotrophy experienced, as many corals can lose their zooxanthellae during periods of stress. During these periods, it might be expected that corals would become more heterotrophic and perhaps as a result the $\delta^{13}C$ of the coral tissue would become more negative. In this regard, a further explanation for the changes in the $\delta^{13}C$ of the respiratory CO_2 and the organic tissues may be that during periods of more negative $\delta^{13}C$, the corals derive more of their food from heterotrophy. In these situations, the $\delta^{13}C$ of the respiratory CO_2 would be closer to that of the zooplankton, which in the Florida Keys is approximately -20 to $-22‰$ (Lutz, 1997). In contrast, during periods of autotrophy the tissues would become more positive in their $\delta^{13}C$ values as they incorporated material translocated from the zooxanthellae.

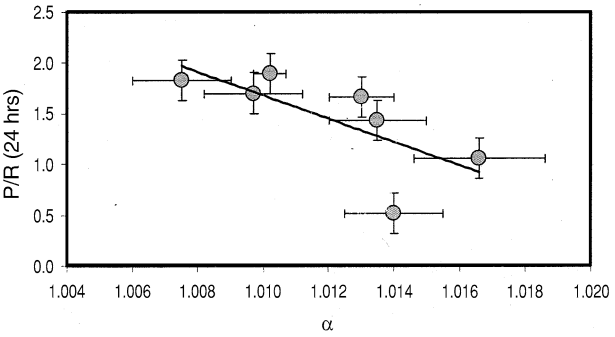

Fig. 7. Correlation between the fractionation factor (α) and the 24-h P/R ratio. The correlation coefficient is 0.71 and statistically significant at the 95% confidence limits. The one sample which falls off the line corresponds to the incubation in September 1993. Without this sample the regression coefficient is 0.90. The error bars represent ± 1 SD of the P/R ratio and α values determined using data obtained from the three corals.

4.6.3. CO_2 limitation

It has been suggested that carbon limitation during periods of high rates of photosynthesis might lead to variation in the $\delta^{13}C$ of coral tissue (Muscatine et al., 1989). At pH values common in seawater ($\sim$ 8.2), most of the dissolved inorganic carbon (ΣCO_2) pool exists as HCO_3^- ($\sim$2.2 mM) whereas the contribution from CO_2 is small ($\sim$20 μM). In the absence of a CO_2-concentrating mechanism, direct uptake of CO_2 from the small pool available in seawater is not sufficient to supply high rates of algal photosynthesis (Muscatine et al., 1984, Weis et al., 1989). Under such conditions it might be expected that fractionation of the stable isotopes would be minimal. The isotopic composition of fixed organic carbon therefore would be isotopically more positive, and the net fractionation (α_i) lower, than that formed under conditions where CO_2 was not limited. This explanation is consistent with seasonal variation observed in the fractionation exerted by the zooxanthellae. Isotopic compositions of fixed photosynthate would be expected to be more positive, when CO_2 utilization by the zooxanthellae would be at its highest level (high P/R values). Conversely, under situations in which CO_2 is not limited, the maximum fractionation between the CO_2 and the zooxanthellae would be expected, such as was observed in August and September, 1993. This pattern is observed in the strong correlation between the P/R ratio and the α_i (Fig. 7). As photosynthate is translocated from the zooxanthellae to the coral, both the carbon isotopic composition of the coral tissues and the respired CO_2 should follow the same trends.

4.6.4. Seasonal loss of zooxanthellae and bleaching

It has been shown that there are seasonal variations in the density of zooxanthellae and chlorophyll in many Caribbean corals, with the lowest values occurring during the late summer months and the highest values occurring during the

winter (Fitt et al., 2000). The species investigated include *Montastraea annularis*, *Acropora palmata*, *A. cervicornis*, and *M. faveolata*. This seasonal loss of zooxanthellae is attributed to the phenomenon of bleaching. These workers hypothesized that all corals exhibit similar patterns in the abundance of zooxanthellae on an annual basis. Changes in the lipid concentrations during bleaching have also been noted by Grottoli et al. (2004). These workers discovered lower proportions of triacylglycerols and wax esters in two species which they investigated. The impact of the loss of zooxanthellae upon the isotopic composition of the coral skeleton has been investigated by several workers with conflicting results (Porter et al., 1989; Leder et al., 1991; Suzuki et al., 2003; Grottoli, 2002; Grottoli et al., in press). Most of these studies (Porter et al., 1989; Suzuki et al., 2003; and Grottoli et al., in press) showed a significant decrease in the $\delta^{13}C$ of the coral as a result of bleaching episodes. However, in the study by Leder et al. (1991) no consistent changes in the $\delta^{13}C$ could be associated with bleaching events. Perhaps some clue as to the reason why different results were obtained lies in the findings of Suzuki et al. (2003) who discovered different responses depending on which side of the coral colony was exposed to light. Samples from the shaded side showed little response during bleaching, whereas exposed sides of the coral exhibited substantial decreases. Nevertheless, regardless of the conclusions of these workers, seasonal variations in the abundance of zooxanthellae would influence the $\delta^{13}C$ of both the respiratory CO_2 and bulk tissues, as observed in this study. During the summer, when the work of Fitt et al. (2000) has shown that zooxanthellae density is at its lowest, it might be expected that the $\delta^{13}C$ of the bulk tissue would be less influenced by photosynthesis and hence be isotopically more negative. During the winter the reverse might be true. Although no measurements of zooxanthellae number were made during this study, our qualitative observations suggest that the corals were already bleaching during late July and August, as temperatures were consistently above 30°C. A final factor which might be of influence is the reproductive activity. During the build-up to the mass spawning event, which occurs during the last full moon in August, the corals build up eggs and sperm in their tissue. This could be expected to influence the isotopic composition of the bulk tissue and the respiratory CO_2. Reproductive activity has been suggested as a mechanism for causing carbon isotopic variation in coral skeletons (Gagan et al., 1994).

4.7. Variations in the Carbon Isotopic Composition of Skeletal Material

The $\delta^{13}C$ of the coral skeleton of corals 8 and 2 varied by ~2.4‰ throughout the course of the experiment (Fig. 5a). The decrease in the skeletal $\delta^{13}C$ occurs during the late summer months approximately concurrent with the decrease in the $\delta^{13}C$ of the respiratory CO_2. However, the magnitude of the seasonal decrease in the $\delta^{13}C$ of the respiratory CO_2 is significantly larger than that recorded in the $\delta^{13}C$ of the skeleton, indicating that as shown by previous studies, the skeleton is formed from mixtures of both respiratory and inorganically derived CO_2. The fact that the changes in the $\delta^{13}C$ of the organic tissues closely mirror that seen in the skeleton necessitates the need to incorporate such variations into future models of carbon isotopic fractionation in coral skeletons, which previously considered the $\delta^{13}C$ of the zooxanthellae and the coral tissues to be relatively constant throughout the year. In this regard, recent papers have suggested that less than 10% of the respiratory CO_2 produced by corals contributes to the isotopic composition of the skeleton (McConnaughey et al., 1997). If correct, this work suggests that any seasonal variation which we observed in the $\delta^{13}C$ of the tissues may be relatively unimportant in controlling the isotopic composition of the skeleton. However, the seasonal variation in fractionation factor (α_i) suggests that the carbon isotopic dynamics of the internal calcification pool are altered seasonally and apparently controlled by uptake of CO_2 by zooxanthellae. As in the case of previous studies by our group (Swart et al., 1996), there appears to be no correlation between either the $\delta^{13}C$ of the coral skeleton and the amount of insolation or the P/R ratio. This can be clearly seen in Figure 8 where before and during the first 2 months of the experiment, the daily light levels were averaging between 30 and 35 E/m^2 (Fig. 8b). The light levels then decreased along with a concomitant decrease in the skeletal $\delta^{13}C$ and P/R ratio (Fig. 8a). The P/R ratios, however, increased again in October 1993 to levels seen earlier in the year, whereas the total amount of light remained low (20 to 10 E/m^2) until April of the following year. The $\delta^{13}C$ of the skeleton, however, increased from its lowest value in October 1993 throughout the remainder of the year until the end of the experiment. At this point, the $\delta^{13}C$ was ~2‰ more positive than at the same time in the previous year, despite experiencing similar light levels and essentially the same P/R ratio. These patterns in the P/R ratio mirror changes in the $\delta^{13}C$ of the ΣCO_2 and therefore suggest a link to the overall productivity of the coral reef ecosystem. Clearly, although there is link between the $\delta^{13}C$ of the coral skeleton and insolation as shown by numerous previous researchers (Weil et al., 1981; Swart et al., 1996; Juillet-Leclerc et al., 1997; Grottoli and Wellington, 1999; Reynard-Vaganay et al., 2001), the timing of variations in insolation mirrors many other changes which take place in the coral reef at the same time, including seasonal variations in the overall productivity of the coral reef, variations in temperature, changes in upwelling patterns, reproduction, and inherent natural cycles in physiology.

5. CONCLUSIONS

The $\delta^{13}C$ of the respiratory CO_2 of *Montastraea faveolata* is during certain portions of the year more negative than the bulk $\delta^{13}C$ of the coral tissue (−13 to −15‰) and the zooxanthellae (−10 to −13‰) and suggests that lipids, which tend to be isotopically depleted compared to other organic compounds, may represent a major portion of the respiratory CO_2.

The $\delta^{13}C$ of both the respiratory CO_2 and the bulk coral tissue show a seasonal pattern with the most positive values occurring during the early portion of the year. More negative $\delta^{13}C$ values occur during the late summer and early winter. Variations in the $\delta^{13}C$ value may reflect seasonal variations in

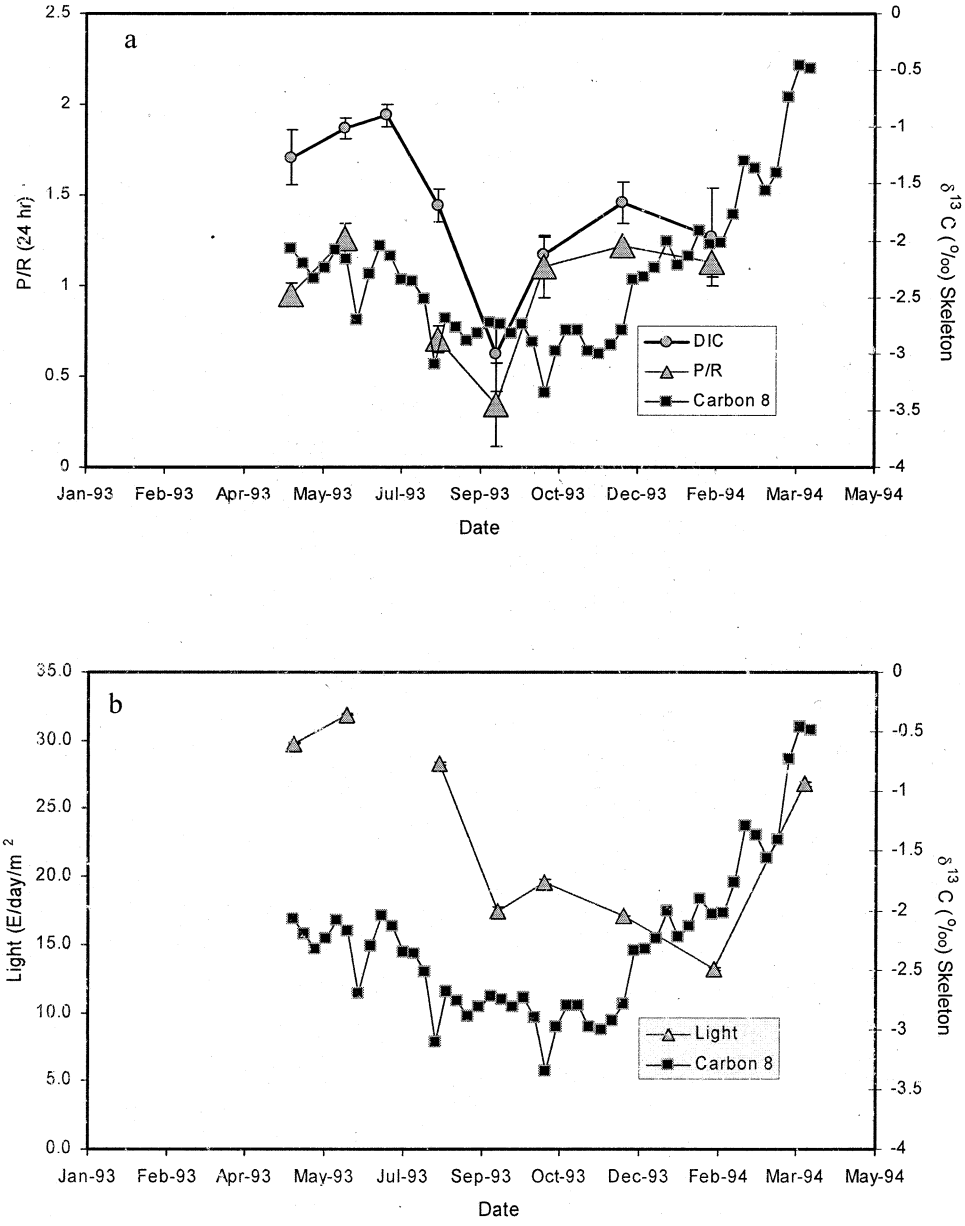

Fig. 8. (a) Changes in the $\delta^{13}C$ of the coral skeleton (coral 8), the $\delta^{13}C$ of the ΣCO_2, and the P/R ratio over the experimental period. The error bars on the P/R ratios represent 1 SD of the P/R values determined for all 18 corals measured during the experimental period. (b) Changes in the $\delta^{13}C$ of the coral skeleton (coral 8) and the mean light. Note that in the case of all the parameters shown there is an approximate correlation between light, P/R, and skeletal $\delta^{13}C$ (coral 8). However, changes in the P/R precede changes in the skeletal $\delta^{13}C$ by several months, whereas the light change is approximately coincident with the change in the skeletal $\delta^{13}C$. In this particular example, the insolation at the beginning and end of the year were approximately the same, but the $\delta^{13}C$ of the skeleton was considerably more positive at the end than at the beginning. This cannot be explained by changes in the $\delta^{13}C$ of the ΣCO_2 or the P/R ratio. This pattern was observed in both the skeletons of the corals analyzed in this study.

the biochemical composition of the coral or changes in the ratio of autotrophy and heterotrophy.

The net fractionation factor experienced during the incorporation of CO_2 by the zooxanthellae is relatively constant (α = 1.0121 $\pm$ 0.003) but appears to show a seasonal variation, with higher values during the summer months.

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APPENDIX 1

Equations describing the change in the $^{13}C/^{12}C$ in a closed volume in response to variations in photosynthesis, respiration, and calcification.

V_{DIC} = Volume of water in incubation
V_{or} = Volume of organic material
V_{sk} = Volume of skeletal material
$^{13}J_{ps}$ = Rate of change of ^{13}C as a result of photosynthesis
$^{13}J_{r}$ = Rate of change of ^{13}C as a result of respiration
$^{13}J_{sk}$ = Rate of change of ^{13}C as a result of skeleton formation
$^{12}J_{ps}$ = Rate of change of ^{12}C as a result of photosynthesis
$^{12}J_{r}$ = Rate of change of ^{12}C as a result of respiration
$^{12}J_{sk}$ = Rate of change of ^{12}C as a result of skeleton formation
$^{13}C_{Or}$ = Concentration of ^{13}C in the organic tissue of the coral and the zooxanthellae
$^{12}C_{Or}$ = Concentration of ^{12}C in the organic tissue of the coral and the zooxanthellae
$^{13}C_{Sk}$ = Concentration of ^{13}C in the skeleton
$^{12}C_{Sk}$ = Concentration of ^{12}C in the skeleton
$^{13}C_{DIC}$ = Concentration of ^{13}C in the ambient DIC
$^{12}C_{DIC}$ = Concentration of ^{12}C in the ambient DIC
k_{ps} = Rate of photosynthesis per unit surface area
k_{r} = Rate of respiration per unit surface area
k_{c} = Rate of calcification per unit surface area
α_i = $^{13}C/^{12}C_{HCO3}/^{13}C/^{12}C_{zooxanthellae}$ (fractionation factor for the incorporation of CO_2 by the zooxanthellae)
α_{ii} = $^{13}C/^{12}C_{CaCO3}/^{13}C/^{12}C_{HCO3}$ (fractionation factor between inorganic $CaCO_3$ and HCO_3^-)
s = surface area of coral

$$\frac{d(V_{DIC}{}^{13}C_{DIC})}{dt} = -{}^{13}J_{ps} + {}^{13}J_{r} - {}^{13}J_{sk} \quad \text{(A1)}$$

$$\frac{d(V_{or}{}^{13}C_{or})}{dt} = {}^{13}J_{ps} - {}^{13}J_{r} \quad \text{(A2)}$$

$$\frac{d(V_{sk}{}^{13}C_{sk})}{dt} = {}^{13}J_{sk} \quad \text{(A3)}$$

$$\frac{d(V_{DIC}{}^{12}C_{DIC})}{dt} = -{}^{12}J_{ps} + {}^{12}J_{r} - {}^{12}J_{sk} \quad \text{(A4)}$$

$$\frac{d(V_{or}{}^{12}C_{or})}{dt} = {}^{12}J_{ps} - {}^{12}J_{r} \quad \text{(A5)}$$

$$\frac{d(V_{sk}{}^{12}C_{sk})}{dt} = {}^{12}J_{sk} \quad \text{(A6)}$$

$${}^{13}J_{Sk} = k_{Sk}\, s \left(\frac{{}^{13}C_{DIC}}{{}^{12}C_{DIC}} - \frac{{}^{13}C_{Sk}}{{}^{12}C_{Sk}\alpha_{ii}} \right) \quad \text{(A7)}$$

$${}^{13}J_{ps} = k_{ps}\, s \left(\frac{{}^{13}C_{DIC}}{{}^{12}C_{DIC}} - \frac{{}^{13}C_{Or}\alpha_{i}}{{}^{12}C_{Or}} \right) \quad \text{(A8)}$$

$${}^{13}J_{r} = k_{r}\, s \frac{{}^{13}C_{or}}{{}^{12}C_{or}} \quad \text{(A9)}$$

$${}^{12}J_{ps} = k_{ps}\, s \quad \text{(A10)}$$

$${}^{12}J_{r} = k_{r}\, s \quad \text{(A11)}$$

$${}^{12}J_{Sk} = k_{Sk}\, s \quad \text{(A12)}$$